Nonalcoholic Fatty Liver Disease: Pathogenesis, Identification, Progression, and Management

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Nonalcoholic fatty liver disease (NAFLD) encompasses a spectrum of liver diseases in the absence of significant alcohol consumption, and its incidence is paralleling the increasing numbers of overweight and obese individuals worldwide. This review discusses the pathogenesis of NAFLD, including the roles potentially played by specific adipokines, such as TNF-α, leptin, and adiponectin. Clinical features, diagnosis, and potential methods of management are also addressed to assist practitioners with the management of this growing population of patients.

Key words: adipokines, management, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis

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INTRODUCTION

The term nonalcoholic steatohepatitis (NASH) was first coined by Ludwig et al.1 in 1980 to describe 20 patients with histologic features of alcoholic liver disease but no history of alcohol abuse. Since this initial description, it has become clear that NASH is but one component of a spectrum of steatotic liver diseases not related to alcohol consumption, commonly referred to as nonalcoholic fatty liver disease (NAFLD).

The spectrum of NAFLD ranges from simple steatosis to NASH, which can then progress to cirrhosis and the development of hepatocellular carcinoma (HCC) in the absence of significant alcohol consumption (Figure 1).2 NASH is differentiated from simple steatosis by the additional presence of necroinflammatory changes and hepatocellular injury.1,3 Thus, the range of pathology within NAFLD must be carefully characterized as simple steatosis, which is a benign entity, with the exception of NASH, which has a very different clinical progression. NASH is now known to be a major cause of cryptogenic cirrhosis, with approximately 20–25% of individuals with these histopathological characteristics developing cirrhosis.4,6 Of those with cirrhosis, 30–40% will experience cirrhosis-related complications including HCC.7,9 Despite its relatively recent discovery, NASH may outpace hepatitis C as the leading cause of liver transplantation by 2025.10,11

PREVALENCE

NAFLD is probably the most common liver disorder worldwide and has emerged as the most frequent cause of abnormal liver enzymes within the United States.12,13 Estimates of NAFLD’s prevalence in the general population ranges from 17% to 33%, rising to 30–100% in the obese population.14–16 In the United States this translates to approximately 30.1 million obese people affected with steatosis and 8.6 million with steatohepatitis.17 Although NAFLD typically presents between the fourth and sixth decades of life, it is known to affect children as well as adults and is not considered discriminatory when it comes to age.18 It also does not discriminate between the sexes, with recent studies demonstrating a similar incidence of NAFLD in men and women.10,19 Among different ethnic groups, however, the picture becomes a bit more complicated—according to recent reports, non-whites comprise the majority of individuals receiving a new diagnosis of NAFLD.20 Hispanics and Asians make up the two largest non-white groups with NAFLD, which introduces the possibility of race-related variability in the susceptibility to NAFLD.20 Furthermore, within specific races, such as Asians, sex-related differences in the prevalence of NAFLD have been observed, which indicates the risk factors for NAFLD may vary depending on ethnicity and sex.20
**RISK FACTORS**

Ludwig et al.’s recognition of an association between NASH and obesity, diabetes, and hyperlipidemia also applies to the entire spectrum of NAFLD. In the obese population, the prevalence of NAFLD increases nearly 5-fold compared to individuals with a normal BMI. Type I and type II diabetes are additional independent risk factors for the development of NAFLD regardless of a person’s weight, and both types add to the severity of the pathology observed. Hyperlipidemia, specifically hypertriglyceridemia, also increases the risk of NAFLD. Increased visceral adiposity, as measured by the waist-to-hip ratio, is also significantly correlated with the degree of hepatic steatosis, highlighting the importance of intra-abdominal fat as a predictor of NAFLD.

Based on these risk factors, it is not surprising that 80% of subjects with NAFLD meet the minimal criteria for the metabolic syndrome. The fundamental pathophysiologic process that connects these diverse conditions is insulin resistance (IR), which appears to be the driving force behind the development of NAFLD.

**PATHOGENESIS OF SIMPLE STEATOSIS TO NASH**

Why some individuals with simple steatosis progress to NASH while others do not remains a story that continues to evolve. Currently, the most widespread and prevailing theory for its progression is the so-called two-hit model, as introduced by Day et al. in 1998. The first hit involves any interference in the metabolism of free fatty acids (FFA) that usually accumulate in the liver. These mechanisms are all heightened in a state of IR. In response to the excess fat in the liver, fatty acids are oxidized and metabolized, releasing reactive oxygen species (ROS) and exposing hepatic cells to a second hit. This release of ROS is likely one of many events promoting the production of oxidative stress and pro-inflammatory cytokines that lead to cell injury and, ultimately, apoptosis and cellular necrosis, which transform a state of simple steatosis to steatohepatitis.

**FREE FATTY ACIDS, INSULIN RESISTANCE, AND VISCERAL ADIPOSE TISSUE**

Intake of excessive calories, fats, and carbohydrates increases free fatty acids within the liver either by increasing delivery or by de novo synthesis. These environmental factors contribute to lipid mobilization and lipolysis, the primary features in the development of IR. Not only is IR common in those with hepatic steatosis, it also perpetuates the development of steatosis by inhibiting the secretion of triglycerides. Normally, FFA are transported to the liver to be degraded in the mitochondria via a process known as mitochondrial beta-oxidation. In the IR state, this process is inhibited, which leads to a buildup of triglycerides within the hepatocytes.

An IR state is closely related to an accumulation of visceral adipose tissue that is linked with unfavorable metabolic consequences. IR and visceral adiposity appear to have a symbiotic relationship, as one perpetuates the other. This occurs as the IR state triggers an increase in lipolysis within visceral adipose tissue, leading to drainage of FFA directly into the portal vein, which results in chronic overload of the liver with high amounts of lipid. Conversely, visceral adipocytes are a depot for the release of various cytokines that have been demonstrated to perpetuate IR. Thus, we can view the process of steatosis development as a continuous cycle that is fueled by the dietary intake and lifestyle of individuals in the 21st century.

**OXIDATIVE STRESS**

Rather than identifying one instigator as the sole cause of the second hit that transforms steatosis to steatohepatitis, it is more likely that a barrage of events...
occurs as the body attempts to adapt to the steatotic environment. Mitochondria, peroxisomes and microsomes participate in this adaptive process by generating oxidative stress via several mechanisms that have been implicated as a main instigator for the progression of steatosis to steatohepatitis.26

One mechanism causing increased oxidative stress is lipid peroxidation of the increased FFA that accumulates in the first hit. A byproduct of this process is the production of ROS, such as hydrogen peroxide, superoxide, and lipid peroxides, which perpetuate further lipid peroxidation.37 Another source of oxidative stress includes the up-regulation of the cytochrome P450 enzymes CYP2E1 and CYP3A4 that provide feedback to increase lipid peroxidation further.25,29 Elevated iron levels represent yet another supply of oxidative potential due to iron’s pro-oxidant activity as well as its association with IR.26,40 However, its true role in precipitating lipid peroxidation and producing ROS remains to be clarified, as existing studies have produced mixed results.31,42 No matter what causes the rise in oxidative stress, this elevation leads to a depletion of the antioxidant pool resulting in a progressive overload of ROS.29

ROLE OF CYTOKINES AND ADIPOKINES

In addition to the products of oxidative stress facilitating the progression of steatosis to NASH, ROS can trigger other hits to catalyze this progression. As briefly mentioned above, visceral adipocytes are now known to secrete cytokines and adipokines. Many of these have been demonstrated to cause dysfunction in lipid metabolism and IR and are thus suspected of being possible mediators in the pathogenesis of NASH.43 One of the cytokines released by adipose tissue as a result of increased ROS is TNF-α.44

TNF-α is a well-known pro-inflammatory cytokine and elevated levels have been demonstrated in NASH subjects.45,46 Further evidence supporting a direct effect on NASH came from experiments in mice that showed TNF-α inhibition led to improvements in liver histology, either through administration of TNF-α Ab or genetically induced TNF-α receptor deficiency.45,46 The relationship between TNF-α and NASH pathogenesis was believed to extend beyond its inflammatory properties as both in vivo and in vitro studies have demonstrated TNF-α activity inducing IR.47,48 However, recent studies that controlled for BMI in humans found no difference in TNF-α levels and the severity of NASH, which raises the question of whether TNF-α truly has an active role in the progression to NASH or is merely a cytokine resulting from obesity.49-51

Leptin is another adipokine that has been investigated as an instigator of NASH. In addition to being a promoter of IR, leptin has been found to affect triglyceride regulation as well as the liver itself.52 Early animal models investigating the relationship between leptin and liver disease were promising, with the results indicating leptin has a profibrotic effect on hepatic stellate cells in murine livers.53,54 However, the results of these animal studies have not translated into conclusive results in human models. Similar to TNF-α, once BMI was controlled for, the association between leptin and both NASH and IR was negated.50,55

Adiponectin is a unique cytokine in that it is the only one found thus far to be inversely related to metabolic dysfunction. This remains true in subjects with NASH, as several studies have observed an inverse relationship between adiponectin and steatosis as well as the degree of inflammation within hepatocytes.49-51,56 It is believed that adiponectin is protective in the liver since it inhibits pro-inflammatory cytokines such as TNF-α and NFκB in vitro.49 Further studies need to be performed to determine the exact role adiponectin may play in the pathogenesis of NASH, but to date, it remains a viable candidate as a marker for the development of NASH. Thus, although adipokines and cytokines initially seemed promising as modifiers of NASH pathogenesis, their true involvement remains to be clarified.

CLINICAL FINDINGS AND DIAGNOSIS

In most cases, NAFLD presents asymptptomatically or is discovered incidentally due to elevated liver-test results or an abnormal liver appearance on ultrasound or abdominal CT scan (Figure 3).26 When symptoms do arise, they are usually vague and may include pain or fullness in the right upper quadrant, fatigue, and general malaise.27 Hepatomegaly is one of the more consistent physical findings, described in up to 75% of patients with NAFLD. Other findings on physical examination that may suggest NAFLD as the cause of liver abnormalities include those characterizing IR and metabolic syndrome, such as central obesity, acanthosis nigricans, hypertriglyceridemia, and hypertension.

The diagnosis of NAFLD requires that two criteria be met: 1) the non-alcoholic nature of the disease must be established and other common causes of liver disease excluded; 2) the presence of fat in the liver must be demonstrated by either imaging studies or liver biopsy. Various studies on the quantification of alcohol consumption have been performed, but the role of alcohol and the amount required to impact liver pathology continues to be debated in the medical literature. Some studies have used a cut-off of 40 g/week, while more recent studies have used a more liberal cut-off of 20 g/day.10,15 In practice, these distinctions are arbitrary and the amount of alcohol consumption is typically assessed
clinically, sometimes requiring interviews with family members of the patient. Ultrasound and CT scan are 75% and 80% sensitive for detecting steatosis, respectively, when there is ≥33% hepatic fat. While radiological studies can establish the diagnosis of NAFLD, no radiological modality is able to distinguish simple steatosis from NASH. Therefore, imaging studies cannot predict the severity of disease and do not take the place of a liver biopsy in establishing the diagnosis of NASH.

Although it is still not possible to diagnose NAFLD based on laboratory testing alone, elevated results of liver tests are found in up to 50% of patients with simple steatosis and in approximately 80% of patients with NASH. A general rule of thumb for confirming a suspicion of NAFLD as a cause of transaminitis is to look for an AST/ALT ratio less than 1, as opposed to an AST/ALT ratio greater than 2, which is suggestive of alcoholic liver disease. In addition, since NAFLD can accompany other liver diseases, it is essential that appropriate laboratory studies be performed to exclude other common causes of liver disease. Research is ongoing to discover a biomarker that can assist physicians in making the distinction between the presence of simple steatosis as opposed to NASH. As described above, adipokine levels in addition to inflammatory markers, such as C-reactive protein and serum ferritin, have been investigated as possible candidates. Unfortunately, none have demonstrated overwhelming reliability.

ROLE OF LIVER BIOPSY AND LIVER HISTOLOGY

Liver biopsy is the only diagnostic test that can reliably identify hepatic steatosis, inflammation, necrosis, and fibrosis; thus, it is the only tool to accurately diagnose NASH in a person with NAFLD. Nevertheless, since liver biopsy is an invasive procedure and carries minor risks, its role in establishing the extent of liver pathology within the spectrum of NAFLD is controversial. The classic argument against liver biopsy is that the findings will not affect patient management since weight loss will ultimately be recommended. Arguments for liver biopsy include the following: 1) alternative non-invasive evaluations have a poor positive predictive value; 2) the correlation between clinical, laboratory, and histological findings for NAFLD is poor; 3) histological staging of NAFLD is the best prognostic indicator. Currently, there is no guideline stipulating when a biopsy is absolutely recommended. However, in clinical practice, a liver biopsy should be considered for patients with risk factors for NAFLD in the setting of persistent transaminitis as well as when an alternative cause of NAFLD not due to IR or metabolic syndrome is suspected and must be ruled out. Furthermore, the proof of steatohepatitis/fibrosis on liver biopsy could affect the decision to proceed with a more aggressive therapeutic option, i.e., bariatric surgery or an experimental pharmacologic treatment for NAFLD.
The histological changes observed in NAFLD are not only limited to the liver parenchyma, they extend into the portal and periportal areas as well. These abnormalities observed on liver biopsy can be divided into three broad categories as follows: steatosis (mild, <1/3; moderate, 1/3–2/3; severe, >2/3); hepatocellular inflammation or injury (ballooning, apoptosis, and necrosis, Mallory’s hyaline, giant mitochondria, polymorphonuclear cells); and fibrosis (perisinusoidal, pericellular). When histology is limited to an observation of steatosis, a diagnosis of simple steatosis is made. However, a combination of steatosis, infiltration by polymorphonuclear cells, hepatocyte ballooning, necrosis, and Mallory bodies distinguish steatohepatitis from simple steatosis. Of these features, hepatocyte ballooning is the most characteristic element for making this distinction. The presence of Mallory’s hyaline is also associated with a diagnosis of steatohepatitis, and immunohistochemical staining for Mallory’s hyaline may assist in distinguishing steatohepatitis when hepatocyte ballooning is in doubt. Fibrosis in NAFLD usually portends a more severe form of liver injury.

As with other forms of viral hepatitis, several grading (activity of inflammation) and staging (degree of fibrosis) systems currently exist for NASH and NAFLD. The two most well-recognized methods are the Brunt system and the NAFLD activity score (NAS). Both of these systems use fatty change, ballooning, and inflammation (parenchymal and portal) to establish the grade of disease. The Brunt system was proposed by Brunt et al. in 1999 to grade and stage NASH specifically rather than the entire spectrum of NAFLD. Although it continues to be a widely used grading tool, limitations include a focus on lobular steatohepatitis versus portal-based disease, its applicability to NASH specifically, and its incorporation of fatty change, ballooning, and inflammation into an overall grade, implying that these characteristics change in parallel. In response, the Nonalcoholic Steatohepatitis Clinical Research Network developed the NAS in 2005 to address these concerns. The NAS gives an individual score to each of the three histological characteristics and the final score is the sum of these three scores. In recent studies, it has proved to be a useful tool for characterizing the range of histological features of NAFLD.

If a decision is made to not proceed with liver biopsy, it is imperative to discuss with the patient the possibility that the extent of liver pathology within the spectrum of NAFLD will remain unclear, even though fatty changes within the liver were shown by the imaging study.

NATURAL HISTORY OF NAFLD

The natural history of NAFLD is currently unknown since only a few prospective studies detailing the histologic follow-up in patients with NAFLD have been conducted. When steatosis is stable without hepatocyte necrosis or fibrosis, there is little potential for histologic or clinical progression. Five studies with histopathologic follow-up data showed histologic stability in 59%, improvement in 13%, and progression of fibrosis or cirrhosis in 28%.

These results were reaffirmed by a recent study that re-evaluated subjects with biopsy-proven NAFLD after a mean follow-up period of 13 years. Their findings demonstrated mortality was not increased in patients with steatosis, but survival of patients with NASH was reduced. These patients experienced greater mortality from cardiovascular and liver-related causes. Moreover, those patients with continued progression of liver fibrosis had the following characteristics in common: more insulin resistance, average weight gain of >5 kg, and greater steatosis at follow-up.

There is mounting evidence that patients with NAFLD may eventually develop cirrhosis and hepatocellular carcinoma (HCC). The prevalence of risk factors associated with NASH may account for the increasing incidence of cryptogenic cirrhosis and subsequent HCC. Obesity and diabetes, per se, are significantly associated with the development of HCC. In particular, diabetes was found to be a risk factor for HCC independent of age, gender, and race. This may stem from chronic hyperinsulinemia and insulin-like growth factor 1 (IGF-1), as studies in human and rat hepatoma cell lines have demonstrated a proliferation of these cells when exposed to physiological insulin concentrations. Furthermore, changes in the expression pattern of IGF-system components has been observed in patients with HCC.

PATIENT MANAGEMENT

The primary goal of therapy when approaching an individual with NAFLD is to provide an intervention that produces a reversal in histological pathology across the entire spectrum of NAFLD. While this is most readily achieved at the stage of simple steatosis, improvements in histology can be expected up to the development of cirrhosis. Once cirrhosis has developed, liver disease due to NAFLD can no longer be reversed. The approach to the management of patients with NAFLD includes the following: 1) correction of the underlying risk factors for NAFLD; 2) avoidance of factors that promote the progression of liver disease; and 3) specific pharmacological treatment for NAFLD.
Correction of Underlying Risk Factors

Because NAFLD is often diagnosed in the setting of insulin resistance or metabolic syndrome, effective treatment of these risk factors is mandatory in patients with NAFLD. Aggressive management of associated hypertension, diabetes, and dyslipidemias should be started promptly. Given the relationship between insulin resistance and NAFLD, an insulin sensitizer is preferred in those with diabetes and NAFLD. It is also safe to use statins to treat hyperlipidemia with careful monitoring of their hepatic side-effect profile.

Since obesity is the most common risk factor for NAFLD, weight reduction plays an integral role in the treatment of NAFLD. There are several diets to choose from, ranging from those advocated by various medical associations (the American Heart Association, ADA, etc.) to commercial enterprises (Atkins, South Beach, etc.). However, the understanding of the specific dietary effects on the treatment of NAFLD remains in an early stage. Recent data has suggested that a high protein/low carbohydrate diet may be more beneficial to obese individuals with characteristics of the metabolic syndrome. This has been supported by the finding, based on food records, that individuals with NAFLD tend to have an excessive intake of energy in the form of carbohydrates and less protein. It has also been suggested that an increased intake of polyunsaturated fats may help reverse histological changes as an imbalance in the ratio of polyunsaturated to saturated fats in subjects with steatosis may promote inflammatory changes within the liver.

Pharmacologic treatment of obesity may be considered in individuals with a BMI > 30 kg/m² or a BMI > 27 kg/m² and associated obesity-related comorbidities. Of the currently available prescription weight-loss medications, two that have been approved for weight management by the FDA (sibutramine and orlistat), have been studied for potential use in NAFLD therapy. In these studies, improvements in liver function tests as well as decreased sonographic evidence of steatosis was noted. However, liver histology was not studied; thus, the use of these drugs for NASH must still be considered experimental.

Bariatric surgery is reserved for those with a BMI > 40 kg/m² or a BMI > 35 kg/m² who are refractory to diet, exercise, or pharmacologic treatments, or for those with obesity-associated comorbidities, e.g., sleep apnea. Although it is very effective for producing excess weight loss, early reports of bariatric surgery detailed a potential exacerbation of steatohepatitis during periods of rapid weight loss (i.e., exceeding 1.6 kg/week). However, more recent trials describe more beneficial effects, with fat, inflammation, and even fibrosis improving following gradual weight loss after gastric bypass.

Avoidance of Factors Promoting Progression of Liver Disease

In a healthy person, it is considered that up to two alcoholic drinks daily are not associated with clinically significant liver damage. However, it is not known whether this holds true for patients with NASH. In the absence of clinical studies, it is prudent to advise patients with a diagnosis of NASH to minimize alcohol consumption. The potential benefits of alcohol on cardiovascular disease are lost when alcohol use is curtailed, but these can potentially be replaced by pharmacologic and lifestyle therapy aimed at minimizing the risk factors for cardiovascular disease. The use of drugs known to cause steatohepatitis, e.g., diltiazem, tamoxifen, steroids, etc., should be avoided in these patients.

Pharmacologic Therapy for NAFLD

Several pharmacologic agents are currently being studied for NAFLD therapy, but those that potentially improve the condition of patients with NAFLD must be examined carefully. The majority of the studies performed to date have been weakened by open-label study designs, small sample sizes, and a lack of uniformity in the diagnoses and outcome assessments. Pharmacological therapy is most commonly directed at various risk factors or pathways for the development of NAFLD. These include weight gain, as described above, modulating insulin resistance, and reducing inflammation (Table 1). Other potential agents that have demonstrated promise in animal models include fatty acid bile acid conjugates, probiotics, and angiotensin-converting enzyme inhibitors.

Table 1. Pharmacological treatment for NASH

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hibitors. These medications, along with those studied in humans, must continue to undergo investigation for efficacy. Until large, randomized, controlled studies with histological follow-up are performed, the following recommendations comprise the preferred strategy for managing patients with NAFLD: exercise, weight control (including dietary modification, pharmacotherapy, and bariatric surgery for qualified patients), treatment of the coexisting conditions (including controlling insulin resistance or diabetes mellitus, controlling hyperlipidemia, and controlling blood pressure), and avoiding alcohol.

CONCLUSION

NAFLD has become a significant public health problem with ties to insulin resistance and the metabolic syndrome. While fatty infiltration of the liver has a benign course, the progression to NASH may lead to cirrhosis and the development of HCC. Standard guidelines for the therapy of NAFLD continue to be a work in progress, with weight loss and lifestyle modification being the mainstays of therapy. Although pharmacological therapy appears promising, more rigorous studies need to be performed before they can be recommended as primary modes of therapy.

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